

Electromyographic changes of motor unit activity in horses with induced hypocalcemia and hypomagnesemia

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Objective—To determine whether electromyographic abnormalities are evident in skeletal muscles in horses with induced hypocalcemia and hypomagnesemia.

Animals—7 healthy adult Dutch Warmblood horses.

Procedures—Electromyographic examination was performed in the lateral vastus, triceps, and subclavian muscles before and after IV infusion of EDTA. An initial dose (mean \pm SD, 564 \pm 48 ml) of a 10% solution of sodium EDTA was administered IV during a period of 21 \pm 7.3 minutes to establish a blood concentration of ionized calcium of approximately 0.5 mmol/L. Average rate of EDTA infusion to maintain ionized calcium at this concentration was 6.6 ml/min.

Results—Mean blood concentrations of ionized calcium and magnesium were 1.39 \pm 0.06 and 0.84 \pm 0.09 mM, respectively before EDTA infusion; after EDTA infusion, concentrations were 0.48 \pm 0.05 and 0.44 \pm 0.20 mM, respectively. This state induced positive waves; fibrillation potentials; doublets, triplets, and multiplets; complex repetitive discharges; and neuromyotonia. Analysis of motor unit action potentials (MUAP) after EDTA infusion revealed an increase in prevalence of polyphasic and complex MUAP in all muscles.

Conclusions and Clinical Relevance—None of the horses had classical signs of hypocalcemia and hypomagnesemia. In contrast, all horses had spontaneous activity in the measured muscles indicative of nerve hyperirritability. Calcium and magnesium deficits appear to have consequences, which may be subclinical, affecting functions of the neuromuscular system. This is of interest for equestrian sports in which hypocalcemia and hypomagnesemia are expected, such as during endurance rides. (*Am J Vet Res* 2002;63:849–856)

Horses subjected to sustained strenuous exercise such as an endurance ride can have substantial losses of fluids and electrolytes.^{1,2} During athletic stress, plasma total calcium concentrations may or may not be reduced, whereas ionized calcium concentra-

tion, which is more accurate proof of hypocalcemia,^{3,4} is often reduced.⁵⁻⁷ This decrease can be induced by calcium loss in sweat, shifting of calcium into RBC or muscles, or increased binding of calcium to albumin or lactate. Furthermore, prolonged aerobic exercise can result in hyperventilation and, consequently, respiratory alkalosis, which further increases the binding of calcium.^{2-4,8}

Hypocalcemia with hypomagnesemia can result in signs of lethargy or may be sufficiently severe to result in death. Synchronous diaphragmatic flutter, tachypnea, stiff gait, muscle fasciculations, profuse sweating and tachycardia, or ataxia are common clinical signs.^{3,7-10} In nerves, lack of calcium has a hyperexcitable effect attributable to an increase in excitability of the axonal membrane^{7,9,11-13} that is a result of a reduction in the depolarization threshold.¹⁴ Hypocalcemia without hypomagnesemia causes paralysis, mydriasis, and stupor.¹⁰

Low calcium concentrations allow sodium to enter nerve cells, which increases nerve irritability resulting in spontaneous contractions and muscle fasciculations.¹⁴⁻¹⁶ In muscles, lack of calcium prevents initiation of muscle contractions that are initiated by calcium release from the sarcoplasmic reticulum and mechanically effectuated through calcium binding to troponin C, which enables formation of actin-myosin complexes.^{14,15,17} Troponin C has 4 receptor sites, 2 of which have high affinity for calcium and 2 for which calcium and magnesium compete.¹⁴ The role of the specific calcium binding site is essential in the process of muscle contraction,¹⁸ whereas magnesium plays a modifying role at this level.¹⁴ Low serum magnesium concentrations are found during tetany caused by strenuous exercise and excessive lactation in mares.^{1,5} Hypomagnesemia potentiates the effects of hypocalcemia⁷ because it increases the release of acetylcholine at neuromuscular junctions.¹⁴ These 2 mechanisms are the basis of the signs of excitation and paresis described in horses with hypocalcemia and hypomagnesemia.

In humans, signs of tetany during electromyographic examination are considered expressions of overexcitability of the peripheral nerves or CNS caused by disturbances in calcium, magnesium, or potassium concentrations.^{7,13,19-21} Electromyographic examination is considered the most sensitive way to investigate tetany because it enables quantification of increased neuromuscular excitability.^{13,21,22}

The study reported here was undertaken to examine electrical activity of the skeletal muscles of horses

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with induced hypocalcemia. To our knowledge, it is the first time that the phenomenon of hypocalcemia in horses has been evaluated by the use of electrophysiologic examination. Rectal temperatures were measured to ensure that none of the horses were hyper- or hypothermic. Intramuscular temperatures were measured because rectal temperatures do not necessarily correlate with IM temperatures, and **electromyography (EMG)** recordings are influenced by temperature.²³⁻²⁵ In addition, the mean concentration of ionized calcium in blood was reduced by administration of EDTA.²⁶ We investigated whether electromyographic examination was able to detect changes in electrical activity of the motor unit in a hypocalcemic state before muscle spasms, a stiff gait or ataxia developed or before the horse became recumbent. In addition, several forms of associated spontaneous activity in the muscles of horses were characterized.

Materials and Methods

Horses—Seven adult Dutch Warmblood horses (4 mares and 3 geldings) were used in the study. Horses were 6 to 10 years old (mean \pm SD, 7.4 ± 1.8 years), weighed between 563 and 690 kg (mean, 630 ± 45 kg), and were 1.62 to 1.69 m tall (mean, 1.66 ± 0.03 m). The horses were owned by the Faculty of Veterinary Medicine and used for educational purposes and riding lessons. They were clinically normal, and they did not have a history of health problems or lameness and had not recently received any medications. Blood biochemical analysis did not reveal abnormalities. The study was approved by the Committee on Animal Welfare of the Faculty of Veterinary Medicine, Utrecht University.

Procedure—Horses were weighed and measured. Horses were then placed in stocks. Rectal temperature^a was measured before EMG recordings were obtained for the normocalcemic state, before EDTA infusion, and after the last EMG recordings. Intramuscular temperature^b of each muscle was obtained after the EMG recordings. Intramuscular temperature was measured by use of local SC administration of a bleb of lidocaine hydrochloride. A probe was then inserted through a 16-gauge 60-mm needle into each muscle, and the probe then was connected to a thermometer.^c

Magnesium concentration in the blood was measured before the start of the experiment and after the last EMG recordings. Muscles on the left side of each horse were used for EMG recordings. One investigator (IDW) performed all EMG recordings^{d,e} and analysis. The lateral vastus muscle and long head of the triceps brachii muscle were chosen for examination because they are important postural muscles that barely generate any **motor unit action potentials (MUAP)** during rest as a result of the passive stay apparatus. Muscle activity during rest was recorded by use of an amplifier gain of 50 μ V/division and sweep speed of 20 ms/division to enable recording of small-amplitude spontaneous activity. Filter settings were 5 Hz and 10 kHz. Sine wave frequencies of muscle action potentials range from 2 Hz to 10 kHz, but a filter setting of 5 Hz was needed to reject background noise. For recording of MUAP, amplifier gain was 100 to 500 μ V/division, depending on the size of obtained MUAP, and sweep speed was 20 ms/division. The notch filter was activated during the recordings. Amplifier gain, filter settings, and sweep speed were chosen on the basis of EMG standards used in humans^{24,27,28} and adapted to our personal preferences and equipment.²⁹

The EMG recordings for the subclavian and triceps muscles were made by use of a disposable concentric 26-gauge EMG needle^f (length, 50 mm; diameter, 0.45 mm; sampling

area, 0.068 mm²). A concentric needle^g (length, 100 mm; diameter, 0.8 mm; sampling area, 0.068 mm²) was used for measurements of the large lateral vastus muscle. A girdle was used to attach a surgical pad to each horse; the surgical pad was connected to the preamplifier and served as the ground electrode.

The MUAP were generated via inducing voluntary muscle contractions by pushing on the horse so that it shifted its weight onto the limb being examined (lateral vastus muscle) or by pulling the limb forward, which provoked the horse to pull back on that limb (triceps muscle). Muscle activity in the subclavian muscle was influenced by shifting the horse's weight onto the left limb.

The first part of the examination was in untreated horses. The EMG recordings started with measurement of insertional activity (3 measurements in various locations within the same muscle). After placement of the needle, it was determined whether spontaneous activity was evident. The needle was inserted in at least 3 locations in each muscle. At each of the 3 locations, the needle was redirected several times. We selected sharp-sounding MUAP during the period when the needle was being withdrawn (3-mm increments) to obtain samples throughout each muscle. The MUAP were partially selected in a semiautomatic way by use of a trigger line that selected identical MUAP above a chosen amplitude. Additional MUAP were selected manually.

Horses were infused with a 10% solution of sodium EDTA,^h and the second part of the study was performed in the same manner as for the first part. The EDTA solution was infused continuously until an ionized calcium concentration of 0.5 mM was achieved. Mean duration for infusion of the volume of EDTA solution required to achieve the desired ionized blood calcium concentration (mean, 564 ± 48 ml) was 21 ± 7.3 minutes. From that point, EDTA solution was administered at a rate of 6.6 ml/min to maintain the ionized calcium concentration. Blood concentration of ionized calcium was measured at 10-minute intervals. Mean total duration of EDTA infusion was 42 ± 7.6 minutes. Mean total time for EMG recording during hypocalcemia was 53 ± 15.1 minutes. Insertional activity, spontaneous activity, and MUAP were recorded during hypocalcemia and analyzed.

Data analysis—Duration of insertional activity was measured semiquantitatively from the beginning of insertion of the needle into the muscle to the end of the activity. Spontaneous activity was recorded when evident. For MUAP analysis, the time for increase of MUAP (ie, risetime) was the duration of the fastest positive (or negative) deflection and was measured automatically. Only MUAP with a maximal risetime of 0.80 milliseconds and that were recorded with an identical superimposed shape at least 4 times were used for statistical analysis.

Amplitude, duration, number of phases, and number of turns were determined. For each superimposed MUAP, the amplitude was measured from the maximum negative to the maximum positive peak values. Duration of MUAP was measured from the initial deflection from baseline to the final return to baseline. Phase was defined as the part of the signal between each crossing of the baseline and was counted as the number of baseline crossings plus 1. A MUAP was classified as polyphasic when it had > 4 phases. Turn was defined as each time the signal changed direction independent of crossing the baseline. A MUAP was classified as complex when it had > 5 turns. Each satellite potential was a late spike that was separate but in close relationship with the main potential.

Fibrillation potentials were defined as spontaneous activity of a single muscle fiber, which sounded similar to rain on a tin roof and had a waveform with an initial positive phase and was recorded outside the end-plate region. Fibrillation potentials were analyzed when they were

detected in 2 or more locations in the same muscle. Positive waves were defined as spontaneous activity of a single muscle fiber, which corresponded to a dull ticking sound and had a waveform with an initial sharp positive deflection followed by a negative wave of longer duration. Doublets, triplets, or multiplets were MUAP that fired repetitively at extremely short intervals (2 to 20 milliseconds). Neuromyotonic discharges were defined as bursts of activity of muscle fibers that fired at extremely high frequencies (> 150 Hz). Complex repetitive discharges were defined as bursts of complex potentials that fired with a fixed amplitude and frequency (20 - 150Hz). Myotonic discharges were characterized as bursts of spontaneous activity with the waveform of positive waves or fibrillation potentials with slight increasing and decreasing amplitudes and firing frequencies. The aforementioned measurements and definitions were determined on the basis of principles defined in human neurophysiologic examinations^{24,28,30} and, when necessary, were adapted on the basis of the authors' experiences with MUAP signals in horses.²⁹

Statistical analysis—Data were normally distributed as determined by use of histograms and the Kolmogorov-Smirnov test for normality. When the data were not normally distributed, transformation of the data into the **natural logarithm (ln)** was performed. Geometric mean values were derived from back transformation of the ln mean values. A 2.5-percentile analysis was calculated because of its independence to distribution of data. Correlations between variables were assessed by use of partial correlation coefficients. A 1-way ANOVA with posthoc Bonferroni testing was performed on transformed data to compare values among muscles and between normo- and hypocalcemic states. The 2-tailed value for significance was set at $P \leq 0.05$.

Results

Ion concentrations—Mean blood concentration of ionized calcium before EDTA infusion was 1.39 ± 0.07 mM. During EDTA infusion, mean minimal ionized calcium concentration was 0.48 ± 0.07 mM (Table 1). Total calcium and magnesium concentrations initially were within reference ranges but decreased during the EDTA infusion. Magnesium concentration decreased significantly from 0.84 ± 0.09 mM before EDTA infusion to 0.44 ± 0.20 mM at the end of EDTA infusion. Potassium concentration decreased significantly ($P = 0.04$) from 3.8 ± 0.41 mM before EDTA infusion to 3.2 ± 0.34 mM after EDTA infusion, but it remained within the reference range (3.0 to 5.9 mM).

Clinical evaluation—Mean heart rate before EDTA infusion was 54 ± 14.2 beats/min, whereas the mean respiratory rate was 27 ± 5.0 breaths/min. At the end of the experiment, mean heart rate had increased significantly ($P = 0.05$) to 64 ± 15.6 beats/min, whereas the mean respiratory rate did not change significantly (26 ± 4.0 breaths/min). Pulse rate peaked at approximately 80 beats/min during the EDTA infusion. Three horses had muscle tremors, pulled their ears backward, or had signs of excitation such as kicking with the hind limbs. None of the horses had classical signs of hypocalcemia such as sweating, tachypnea, synchronous diaphragmatic flutter, a stiff gait, or ataxia.

Temperatures—Intramuscular temperature did not differ significantly among the 3 muscles; mean for

Table 1—Electrolyte concentrations in blood samples of 7 horses before and at the end of infusion of an EDTA solution

| Electrolyte | Minimum | Maximum | Mean | SD |
|----------------------|---------|---------|------|------|
| Ionized calcium (mM) | | | | |
| Before | 1.30 | 1.51 | 1.39 | 0.06 |
| End | 0.34 | 0.57 | 0.48 | 0.05 |
| Magnesium (mM) | | | | |
| Before | 0.74 | 1.02 | 0.84 | 0.09 |
| End | 0.26 | 0.88 | 0.44 | 0.20 |
| Potassium (mM) | | | | |
| Before | 3.5 | 4.7 | 3.81 | 0.38 |
| End | 2.9 | 3.9 | 3.17 | 0.32 |

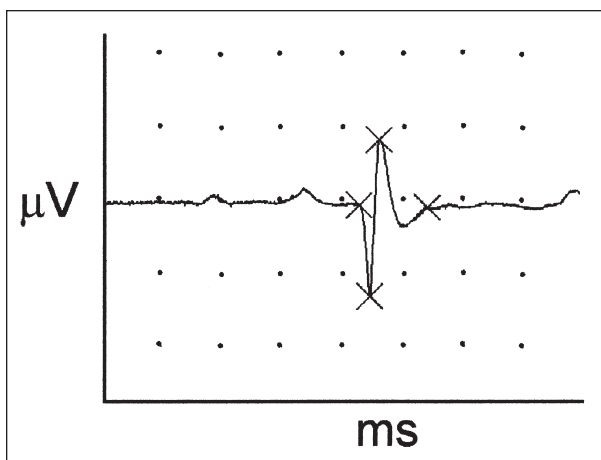


Figure 1—Electromyographic recording obtained from the left subclavian muscle of an adult horse in a normocalcemic state revealing a normal motor unit action potential (MUAP). Each division represents 200 μ V (y-axis) and 5 milliseconds (x-axis). The time for increase of the MUAP (risetime) is 0.5 milliseconds. Notice the 3 phases (defined as the part of the signal between each crossing of the baseline) and 3 turns (defined as each time the signal changed direction independent of crossing the baseline) in the MUAP. Measuring points for MUAP duration and MUAP amplitude are indicated (X).

the 3 muscles was 36.89 ± 0.05 C. Mean temperature in the muscles was significantly ($P = 0.002$) lower than mean rectal temperature (38.13 ± 0.09 C). Mean rectal temperature was lower, but not significantly different, at the end of the experiment. A correlation between the MUAP variables and the temperatures was not found for the triceps muscle, whereas a significant negative correlation ($r = -0.8349$; $P = 0.04$) was found between the number of phases and the rectal temperature at the end of the experiment for the subclavian muscle. In the lateral vastus muscle, a nonsignificant negative correlation ($r = -0.9189$; $P = 0.10$) was found between the number of phases and the rectal temperature, but a significant negative correlation ($r = -0.9796$; $P = 0.001$) was found between number of phases and temperature in the muscle.

MUAP analysis—Insertional activity was not altered during the hypocalcemic state. Geometric mean amplitude, duration, number of phases, and number of turns of the MUAP (Fig 1) also were not significantly altered by hypocalcemia, except for the number of phases and turns in the subclavian muscle, which increased significantly (Table 2). In addition, the percentages of polyphasic and complex MUAP increased significantly in all 3 muscles (Fig 2 and 3). In the sub-

Table 2—Variables for analysis of motor unit action potentials (MUAP) obtained during induced hypocalcemia and hypomagnesemia in 7 horses

| Variable | Muscle | gMean | SD | 95% CI | Range |
|---------------------------------------|------------------|-------|------|-----------|------------|
| Duration of insertional activity (ms) | All* | 707 | 246 | 592–822 | 316–1,262 |
| Amplitude (μ V) | Subclavian | 295 | 2.84 | 264–331 | 37–9,799 |
| | Triceps | 341 | 3.82 | 282–413 | 54–10,199 |
| | Vastus lateralis | 388 | 2.90 | 340–447 | 86–9,605 |
| Duration (ms) | Subclavian | 7.29 | 1.61 | 6.77–7.86 | 2.01–38.09 |
| | Triceps | 6.25 | 2.13 | 5.52–7.07 | 2.02–38.09 |
| | Vastus lateralis | 6.02 | 2.30 | 5.41–6.70 | 1.99–39.64 |
| No. of phases | Subclavian | 2.83† | 1.43 | 2.72–2.94 | 1–7 |
| | Triceps | 2.44 | 1.45 | 2.29–2.59 | 1–7 |
| | Vastus lateralis | 2.59 | 1.45 | 2.46–2.69 | 1–20 |
| No. of turns | Subclavian | 3.42† | 1.63 | 3.22–3.60 | 1–20 |
| | Triceps | 3.07 | 1.63 | 2.83–3.33 | 1–11 |
| | Vastus lateralis | 3.15 | 1.30 | 2.97–3.39 | 1–20 |

*Represents mean \pm SD value for subclavian, triceps, and vastus lateralis muscles. †Represents significant increase, compared with the normocalcemic state.

95% CI = 95% Confidence interval. gMean = Geometric mean.

Phase was defined as the part of the signal between each crossing of the baseline and was counted as the number of baseline crossings plus 1. Turn was defined as each time the signal changed direction independent of crossing the baseline.

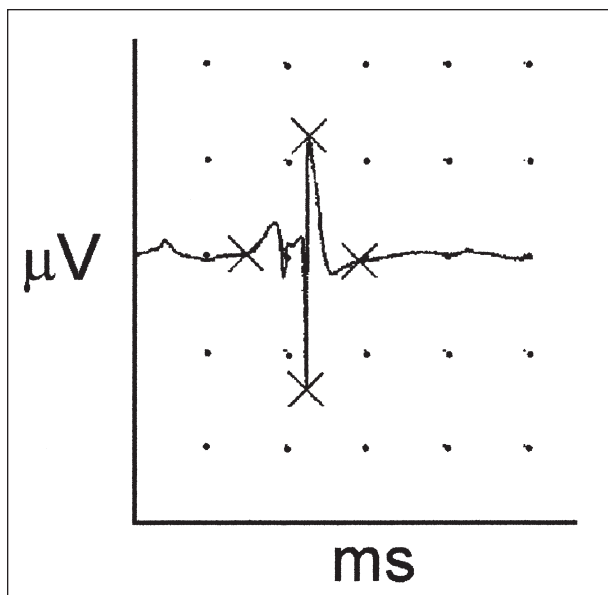


Figure 2—Electromyographic recording obtained from the left lateral vastus muscle of an adult horse in a hypocalcemic and hypomagnesemic state. Notice the polyphasic and complex MUAP, 6 phases, and 8 turns. Each division represents 200 μ V (y-axis) and 5 milliseconds (x-axis). The risetime is 0.1 milliseconds. Measuring points for MUAP duration and MUAP amplitude are indicated (X).

clavian muscle, the percentage of polyphasic MUAP almost doubled (from 6.2 to 11.9%), and the percentage of complex MUAP increased from 2.6 to 16.8%. For the triceps muscle, the percentage of polyphasic MUAP increased from 3.9 to 7.6%, whereas the percentage of complex MUAP increased from 5.7 to 13.2%. In the lateral vastus muscle, the percentage of complex MUAP increased from 9.6 to 13.2%.

In the normocalcemic state, satellite potentials were not detected in the triceps muscle, but in the sub-

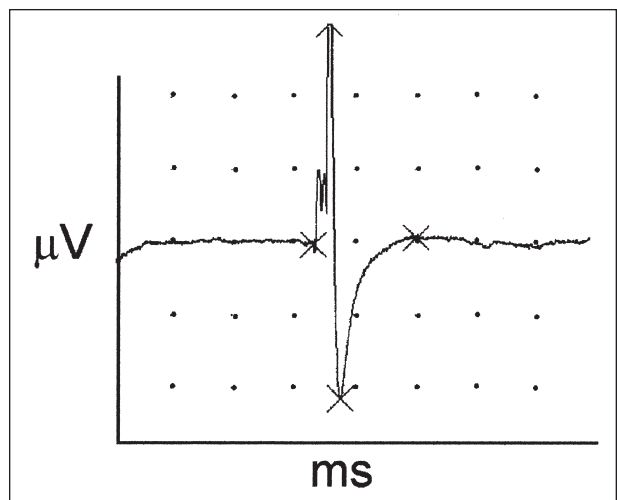


Figure 3—Electromyographic recording obtained from the long head of the left triceps brachii muscle of an adult horse in a hypocalcemic and hypomagnesemic state. Notice the complex MUAP, 3 phases, and 7 turns. Each division represents 200 μ V (y-axis) and 5 milliseconds (x-axis). The risetime is 0.2 milliseconds. Measuring points for MUAP duration and MUAP amplitude are indicated (X).

clavian muscle, 2.9% of the MUAP were accompanied by satellite potentials (3/7 horses). In the lateral vastus muscle, satellite potentials accompanied 1.4% of the MUAP (1/7 horses). Satellite potentials were not detected in any of the muscles during the hypocalcemic state.

Significant correlations were detected during the hypocalcemic state between number of phases and number of turns in the triceps ($r = 0.8660$; $P = 0.026$) and lateral vastus ($r = 0.8254$; $P = 0.043$) muscles. The correlation between duration and amplitude in the lateral vastus muscle ($r = 0.9423$; $P = 0.05$) during the hypocalcemic state differed from the correlation during the normocalcemic state ($r = 0.9610$; $P = 0.002$).

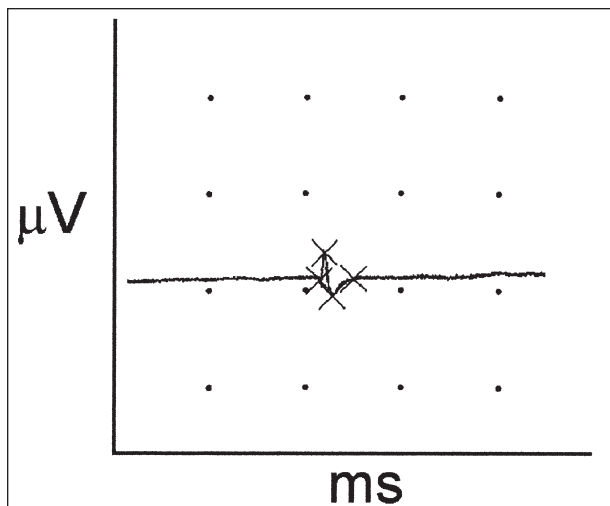


Figure 4—Electromyographic recording obtained from the long head of the left triceps brachii muscle of an adult horse in a hypocalcemic and hypomagnesemic state. Notice the fibrillation potentials. Each division represents 200 μ V (y-axis) and 5 milliseconds (x-axis). Measuring points for MUAP duration and MUAP amplitude are indicated (X).

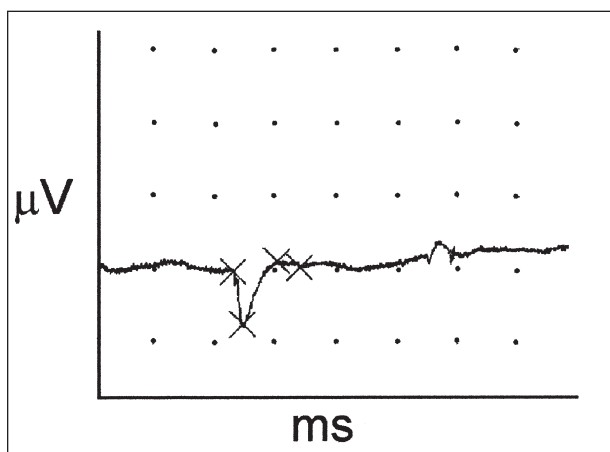


Figure 5—Electromyographic recording obtained from the left subclavian muscle of an adult horse in a hypocalcemic and hypomagnesemic state. Notice the positive sharp waves. Each division represents 100 μ V (y-axis) and 5 milliseconds (x-axis). Measuring points for MUAP duration and MUAP amplitude are indicated (X).

Correlation between number of phases and number of turns in the lateral vastus muscle during the hypocalcemic state ($r = 0.8254$; $P = 0.043$) was comparable to the correlation during the normocalcemic state. We did not detect a significant correlation between variables for the subclavian muscle during the hypocalcemic state.

Spontaneous activity—During normocalcemia, sporadic spontaneous activity was registered in the triceps (myotonic discharge) and lateral vastus (positive wave) muscles of 1 horse. During the hypocalcemic state, spontaneous activity was encountered abundantly in each muscle in all horses. Fibrillation potentials, positive waves, and doublets were detected in 21 of 21 muscles (3 muscles/horse \times 7 horses), and neuromyotonia was

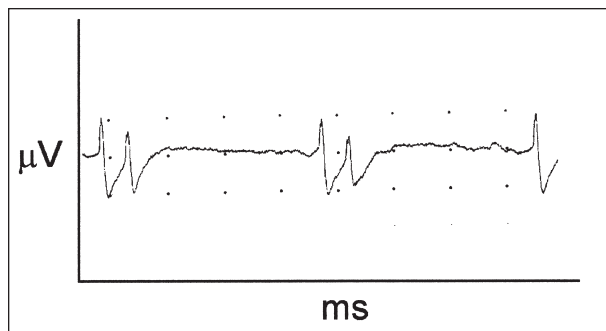


Figure 6—Electromyographic recording obtained from the long head of the left triceps brachii muscle in an adult horse in a hypocalcemic and hypomagnesemic state. Notice the doublets. Each division represents 100 μ V (y-axis) and 10 milliseconds (x-axis).

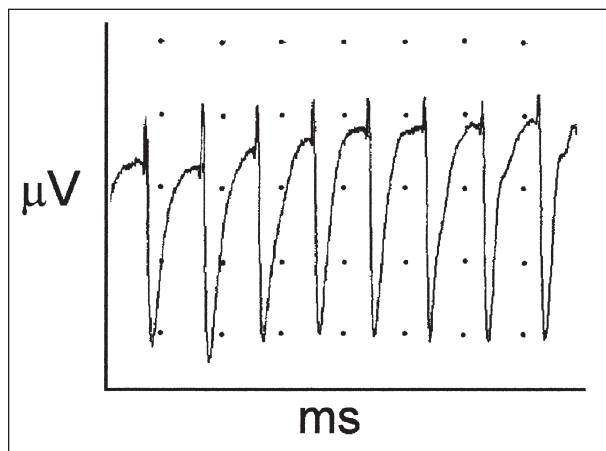


Figure 7—Electromyographic recording obtained from the long head of the left triceps brachii muscle of an adult horse in a hypocalcemic and hypomagnesemic state. Notice the neuromyotonia. Firing frequency was 200 Hz. Each division represents 100 μ V (y-axis) and 5 milliseconds (x-axis).

detected in 20 of 21 muscles (Fig 4–7); an exception was the lateral vastus muscle of 1 horse. Spontaneous activity consisting of repetitive-firing MUAP (doublets, 21/21; triplets, 18/21; quadruplets, 11/21; and multiplets, 9/21) was detected frequently. Myotonic (11/21) and complex repetitive (12/21) discharges were encountered less frequently. Myotonic discharges comprising fibrillation potentials and positive waves were recorded.

Characteristics of the fibrillation potentials and positive waves were determined (Table 3). Values for amplitude and duration were normally distributed. Duration of fibrillation potentials found in the lateral vastus muscle was significantly ($P = 0.002$) shorter than in the other muscles. Furthermore, amplitude of fibrillation potentials in the subclavian muscle was significantly ($P = 0.02$) higher, compared with values for the other muscles. Positive waves were similar in the 3 muscles, except that the duration was shortest in the lateral vastus muscle and differed significantly ($P = 0.001$) from the duration for the subclavian muscle. Mean firing frequencies for neuromyotonic, myotonic, and complex repetitive discharges were 196.98 ± 35.60 , 128.45 ± 22.40 , and 140.33 ± 13.02 Hz, respectively.

Table 3—Variables of fibrillation potentials and positive waves in 3 muscles of horses in a hypocalcemic and hypomagnesemic state after infusion of EDTA solution

| Variable | Muscle | Mean | SD | 95% CI | Range |
|---|------------------|-------|-------|-------------|------------|
| Fibrillation potential amplitude (μ V) | Subclavian | 54.51 | 21.36 | 52.17–56.85 | 12–140 |
| | Triceps | 52.79 | 20.20 | 45.95–52.61 | 15–125 |
| | Vastus lateralis | 49.28 | 20.88 | 50.42–55.16 | 14–155 |
| Duration (ms) | Subclavian | 3.84 | 1.36 | 3.69–3.99 | 1.12–10.10 |
| | Triceps | 3.60 | 1.04 | 3.43–3.78 | 1.80–7.10 |
| | Vastus lateralis | 3.18 | 1.03 | 3.06–3.29 | 1.06–7.74 |
| No. of phases | Subclavian | 2.25 | 0.56 | 2.19–2.31 | 1–4 |
| | Triceps | 2.24 | 0.47 | 2.16–2.31 | 1–3 |
| | Vastus lateralis | 2.26 | 0.55 | 2.10–2.23 | 1–4 |
| No. of turns | Subclavian | 2.32 | 0.63 | 2.25–2.39 | 1–5 |
| | Triceps | 2.22 | 0.52 | 2.14–2.31 | 1–4 |
| | Vastus lateralis | 2.26 | 0.61 | 2.19–2.33 | 1–4 |
| Positive wave amplitude (μ V) | Subclavian | 67.63 | 44.98 | 56.30–78.96 | 22–261 |
| | Triceps | 77.35 | 44.24 | 65.15–89.54 | 19–215 |
| | Vastus lateralis | 66.21 | 39.60 | 57.16–75.26 | 13–207 |
| Duration (ms) | Subclavian | 5.50 | 2.38 | 4.90–6.10 | 2.14–12.78 |
| | Triceps | 5.09 | 1.86 | 4.57–5.60 | 2.34–10.08 |
| | Vastus lateralis | 4.35 | 1.55 | 4.70–4.29 | 1.46–8.40 |
| No. of phases | Subclavian | 1.54 | 0.59 | 1.39–1.69 | 1–3 |
| | Triceps | 1.42 | 0.50 | 1.28–1.55 | 1–2 |
| | Vastus lateralis | 1.36 | 0.48 | 1.25–1.47 | 1–2 |
| No. of turns | Subclavian | 1.63 | 0.70 | 1.46–1.81 | 1–4 |
| | Triceps | 1.66 | 0.88 | 1.42–1.90 | 1–4 |
| | Vastus lateralis | 1.64 | 0.80 | 1.46–1.83 | 1–5 |

Discussion

Four of 7 horses in the study reported here did not have specific clinical signs of hypocalcemia. Thus, this study revealed that changes in functions of the neuromuscular system in hypocalcemic and hypomagnesemic horses can be detected that are not associated with specific clinical signs such as synchronous diaphragmatic flutter, dysphagia, stiff gait of the hind limbs, goose-stepping, convulsions, or ataxia. The respiratory rate was higher than in clinically normal horses; however, ambient temperatures were high (approx 25 C). Furthermore, the horses were involved in EMG recordings for a prolonged period, and the electromyographic examination generated sounds that were quite extreme and loud, especially during EDTA infusion. Heart rate increased during the EDTA infusions, but pulse frequency and respiratory rate, which are expected to be higher after exercise, were not. Therefore, pulse frequency and respiratory rate may not be suitable variables to evaluate in horses for use as indicators of hypocalcemia and hypomagnesemia during periods of strenuous exercise.

Horses respond to hypocalcemia in a manner similar to that of dogs and humans, and the effect of nervous irritability apparently overrules the hypocalcemia-induced reduced release of acetylcholine.^{16,17} This release is mediated by calcium-dependent calmodulin activation of neurotransmitter.³¹ Tetanic contractions develop when concentrations of total calcium are between 1.25 and 2 mM, and total calcium concentrations < 1.25 mM result in paresis. The blood concentration of ionized calcium that was induced in

the horses of our report (approx 0.5 mM) is well within the range at which abnormalities can be expected.^{5,9}

The fact that magnesium concentrations in our horses were reduced by half (to 0.44 mM) after EDTA infusion is important, because in studies^{13,22} in humans, hyperexcitability is also attributed to the effect of decreased intracellular magnesium concentrations on hypocalcemia-induced hyperirritability. Magnesium blocks the release of acetylcholine from vesicles at the site of the neuromuscular junction that results from depolarization-induced calcium influx into the nerve terminals.^{12,13,15} Therefore, hyperirritability is enhanced when magnesium concentrations are low. Concomitant hypomagnesemia is associated with hypocalcemia in humans and horses,^{5,7,12,13} and concentrations of magnesium between 0.23 and 0.48 mM (instead of 0.63 mM) have been reported in hypocalcemic horses.^{5,9} It is interesting that the magnesium concentrations in blood did not change in calves with EDTA-induced hypocalcemia.³² Potentially, potassium can also influence results of EMG during tetany in humans^{13,21} by enhancing nervous irritability¹⁴; however, potassium concentrations remained within reference ranges in our horses.

The EMG recordings revealed an emphasis on an increase in spontaneous activity with small changes in MUAP configurations. Mean number of phases and number of turns increased only in the subclavian muscle. This indicated an increase in temporal dispersion^{28,33} of the discharges for a single muscle fiber that contribute to the MUAP^{23,28} and corresponded with the increase in polyphasic and complex MUAP³⁴ found in

all muscles. Little information on quantitative MUAP analysis is available, and it is not yet known which normative data are most suitable to discriminate clinically normal individuals from individuals with disease. The negative correlation found between the temperatures in the subclavian and lateral vastus muscles and the number of phases may be explained by an increase in temporal dispersion when the temperature decreases.^{23,25,33}

Of the various forms of spontaneous activity that were recorded during the hypocalcemic and hypomagnesemic state in our study, neuromyotonia as well as doublets, triplets, quadruplets, and multiplets are specifically indicative of neurogenic hyperexcitability.^{30,35,36,i} On the other hand, fibrillation potentials and positive waves are evident in neurogenic and myogenic disorders.^{30,35,36,i} Specific EMG findings in hypocalcemic humans included doublets, triplets, and multiplets^{13,19,21,22} as well as neuromyotonia²⁴ in addition to other repetitive discharges that were not specified.^{13,19,i} We did not detect specific EMG findings described in hypomagnesemic humans, other than the fact that hypomagnesemia can also induce tetany in men.²⁰

The induction of various spontaneous activity provided us the opportunity to study spontaneous activity and statistically describe fibrillation potentials and positive waves in horses. Many studies³⁷⁻³⁹ on EMG in horses mention spontaneous activity but lack an objective description, whereas another study⁴⁰ referred only to reference values in humans. The duration of fibrillation potentials and positive waves in the horses of the study reported here appeared to be shorter than in humans.^{30,35,41} Differences among muscles for the morphologic characteristics of fibrillation potentials that were found in the horses of our study have not been described in humans.

Clinical signs indicative of electrolyte changes induced by EDTA infusion may not be the same as those resulting from exercise-induced electrolyte loss. However, the induced electrolyte changes were comparable to changes during tetany resulting from strenuous exercise.⁶ The EDTA-infusion model revealed that hypocalcemia can induce subclinical alterations in the excitability of motor neurons. The EMG abnormalities consisted of various forms of specific neurogenic spontaneous activity (doublets, triplets, quadruplets, and multiplets as well as neuromyotonia) together with nonspecific myogenic spontaneous activity (fibrillation potentials, positive waves) without major changes in MUAP variables. Furthermore, EDTA-induced hypocalcemia appeared to be effective for use in inducing and analyzing various forms of spontaneous activity, which resulted in objective normative data for the horses of our report.

^aModel 8011 Fritz thermometer, Vetin-Aacofarma, Bostel, The Netherlands.

^bVoltcraft 500, Conrad Electronic, Berlin, Germany.

^cVoltcraft 500 K-type thermocouple, Conrad Electronic, Berlin, Germany.

^dNicolet Meridian EMG apparatus, Nicolet Biomedical Inc, Madison, Wis.

^eTopline 8000, Topline, Hoevelaken, The Netherlands.

^fEMG concentric needle electrode, Nicolet Biomedical Inc, Madison, Wis.

^gEMG concentric needle electrode, Nicolet Biomedical Inc, Madison, Wis.

^hSodium EDTA 10%, Veterinary Pharmacy, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

ⁱPoenaru S, Stanescu V. The EMG test in the study of pseudo-hypoparathyroidism and pseudo-pseudo-hypoparathyroidism (abstr). *Electroencephalogr Clin Neurophysiol* 1968;25:412.

References

1. Flaminio JBF, Rush BR. Fluid and electrolyte balance in endurance horses. *Vet Clin North Am Equine Pract* 1998;14:147-158.
2. White SL. Fluid, electrolyte, and acid-base balances in three-day, combined-training horses. *Vet Clin North Am Equine Pract* 1998;14:137-145.
3. Carlson GP. Clinical chemistry tests. In: Smith BP, ed. *Large animal internal medicine*. 2nd ed. St Louis: CV Mosby Co, 1996; 453-455.
4. Lincoln SD, Lane VM. Serum ionized calcium concentration in clinically normal dairy cattle and changes associated with calcium abnormalities. *J Am Vet Med Assoc* 1990;197:1471-1474.
5. Brewer BD. Disorders of equine calcium metabolism. *Compend Contin Educ Pract Vet* 1982;4:s244-s251.
6. Carlson GP, Mansmann RA. Serum electrolyte and plasma protein alterations in horses used in endurance rides. *J Am Vet Med Assoc* 1974;165:262-264.
7. Smith KA, Wagner PC. Electrolyte imbalances and metabolic disturbances in endurance horses. *Compend Contin Educ Pract Vet*. 1985;7:s575-s585.
8. Mansmann RA, Carlson GP, White NA, et al. Synchronous diaphragmatic flutter in horses. *J Am Vet Med Assoc* 1974;165:265-270.
9. Brandt GW. Hypocalcaemic tetany. In: Robinson NE, ed. *Current therapy in equine medicine* 3. Philadelphia: WB Saunders Co, 1992;566-569.
10. Reef V. Arrhythmias. In: Marr CM, ed. *Cardiology of the horse*. London: WB Saunders Co, 1999;206.
11. Lentz LR, Valberg SJ, Balog EM, et al. Abnormal regulation of muscle contraction in horses with recurrent exertional rhabdomyolysis. *Am J Vet Res* 1999;60:992-999.
12. Sivak M, Ochoa J, Frenandez JM. Approaches to the nervous system in electromyography. In: Brown WF, Bolton CF, eds. *Clinical electromyography*. Boston: Butterworth-Heinemann, 1993;136-137.
13. Starita A, Giusti A, Rossi B, et al. Evaluation of the EMG spontaneous activity in tetany: further development of a method of automatic analysis. *Electromyogr Clin Neurophysiol* 1984;24:619-623.
14. Gordon AM. Molecular basis of contraction. In: Patton HD, Fuchs AF, Hille B, et al, eds. *Textbook of physiology: excitable cells and neurophysiology*. 21st ed. Philadelphia: WB Saunders Co, 1989; 177-178.
15. Breazile JE. Skeletal muscle physiology. In: Swenson MJ, Reece WC, eds. *Duke's physiology of domestic animals*. 11th ed. Ithaca, NY: Comstock Publishing Association, 1994;855-873.
16. Coffman J. Calcium and phosphorus physiology and pathophysiology. *Equine Pract* 1980;9:93-96.
17. Iggo A. Activity of peripheral nerves and junctional regions. In: Swenson MJ, ed. *Duke's physiology of domestic animals*. 10th ed. Ithaca, NY: Comstock Publishing Associates, 1983;613-614.
18. Ward TL, Valberg SJ, Gallant EM, et al. Calcium regulation by skeletal muscle membranes of horses with recurrent exertional rhabdomyolysis. *Am J Vet Res* 2000;61:242-247.
19. Jableck CK. Disorders of neuromuscular transmission and muscles. In: Brown WF, Bolton CF, eds. *Clinical electromyography*. Boston: Butterworth-Heinemann, 1993;677-678.
20. Kimura J. Neuromuscular diseases characterized by abnormal muscle activity. *Electrodiagnosis in diseases of nerve and muscle: principles and practice*. 2nd ed. Philadelphia: FA Davis Co, 1989;567.
21. Lorenzoni E. The diagnostic possibilities of the EMG in tetany. *Electroencephalogr Clin Neurophysiol* 1969;27:211-214.
22. Rossi B, Sartucci F, Starita A. Automatic analysis of the spontaneous EMG activity during ischaemic test in tetany. *Electromyogr Clin Neurophysiol* 1984;24:75-80.
23. Buchthal F, Pinelli P. Action potentials in normal muscle. *Acta Physiol Scand* 1951;25(suppl 89):13-14.
24. Daube JR. American Association of Electromyography and

Electrodiagnosis minimonograph No. 11. Needle examination in clinical electromyography. *Muscle Nerve* 1991;14:685-700.

25. Ricker K, Hertel G, Stodieck G. Increased voltage of the muscle action potential of normal subjects after local cooling. *J Neurol* 1977;216:33-38.

26. Aquilera-Tejero E, Garfia B, Estepa JC, et al. Effects of exercise and EDTA administration on blood ionized calcium and parathyroid hormone in horses. *Am J Vet Res* 1998;59:1605-1607.

27. Kimura J. Electrodes and recording apparatus. *Electrodiagnosis in diseases of nerve and muscle: principles and practice*. 2nd ed. Philadelphia: FA Davis Co, 1989;41-43.

28. Stålberg E, Nanedkar SD, Sanders DB, et al. Quantitative motor unit potential analysis. *J Clin Neurophysiol* 1996;13:401-422.

29. Wijnberg ID, Franssen H, van der Kolk JH, et al. Quantitative motor unit action potential analysis of the normal subclavian muscle in horses. *Am J Vet Res* 2002;63:198-203.

30. Oh SJ. Needle electromyography study. *Principles of clinical electromyography case studies*. Baltimore: The Williams & Wilkins Co, 1998;77-119.

31. Iggo A, Klemm WR. Nerves, junctions, and reflexes. In: Swenson MJ, Reece WC, eds. *Duke's physiology of domestic animals*. 11th ed. Ithaca, NY: Comstock Publishing Association, 1994;780-784.

32. Desmecht DJ, Linden AS, Godeau JM, et al. Experimental production of hypocalcemia by EDTA infusion in calves: a critical appraisal assessed from the profile of blood chemicals and enzymes. *Comp Biochem Physiol A Physiol* 1995;110:115-130.

33. Steinberg HS. A review of electromyographic and motor nerve conduction velocity techniques. *J Am Anim Hosp Assoc* 1979;15:613-619.

34. Feinstein B, Lindegård B, Nyman E, et al. Studies on action potentials in normal human muscles. *Acta Psychiatr Neurol Scand* 1951;29:189-195.

35. Daube JR. Electrophysiologic studies in the diagnosis and prognosis of motor neuron diseases. *Neurol Clin* 1985;3:473-493.

36. Emeryk B, Hausmanowa-Petrusewicz I, Nowak T. Spontaneous volleys of bizarre high-frequency potentials (b.h.f.p.) in neuro-muscular diseases. Part II. Analysis of the morphology of spontaneous volleys of bizarre high frequency potentials in neuro-muscular diseases. *Electromyogr Clin Neurophysiol* 1974;14:339-354.

37. Naylor JM, Jones V, Berry SL. Clinical syndrome and diagnosis of hyperkalaemic periodic paralysis in Quarter horses. *Equine Vet J* 1993;25:227-232.

38. Spier SJ, Carlson GP, Holliday TA, et al. Hyperkalemic periodic paralysis in horses. *J Am Vet Med Assoc* 1990;197:1009-1017.

39. Valentine BA, de Lahunta A, George C, et al. Acquired equine motor neuron disease. *Vet Pathol* 1994;31:130-138.

40. Andrews, FM. Electrodiagnostic aids and selected neurological diseases. In: Reed SM, Bayly WM, eds. *Equine internal medicine*. Philadelphia: WB Saunders Co, 1998;438-447.

41. Sethi RK, Thompson LL. The EMG examination. *The electromyographer's handbook*. 2nd ed. Boston: Little, Brown & Co, 1989;126-150.